Assessment of the histological quality of endoscopic biopsies obtained from the canine gastro-esophageal junction

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Key words
Esophagoscopy, histology, esophagus, stomach, z-line, biopsy technique

Summary
Objective: In the dog biopsy samples from the gastro-esophageal junction (GEJ) are rarely obtained during routine gastroscopy. The aim of this pilot study was to assess the histological quality of endoscopic biopsies sampled from the canine esophagus and cardia. It was hypothesised that it is possible to sample adequate specimens from these sites. Materials and methods: For this purpose 10 dogs with an indication for gastroscopy were enrolled in a prospective study. Biopsy samples were obtained with standard biopsy forceps for single use exactly from the GEJ thus containing preferably columnar epithelium from the cardia and squamous epithelium from the esophagus, respectively. In every dog the specimens were examined for size, layers and site, respectively. Study endpoint was reached when specimens originated from cardia and esophagus, showing at least epithelium and lamina propria mucosae, and a diameter > 2 mm on the slide, respectively. Results: 72 biopsy specimens (median 7, range 5–10) obtained from the GEJ were examined in 10 dogs. Specimens from the esophagus containing squamous epithelium with lamina propria mucosae were found in 5 of 10 (50.0%) dogs. Specimens from the cardia containing columnar epithelium with lamina propria mucosae were found in 10 of 10 (100.0%) dogs. Four of 10 (40.0%), and 10 of 10 (100.0%) dogs showed at least one specimen > 2 mm on the slide originating from the esophagus, and from the cardia, respectively. Histological quality was found to be adequate in 4 of 10 (40.0%) dogs, showing specimens of adequate size, originating from both esophagus and cardia, and containing at least epithelium and lamina propria mucosae. Conclusion and clinical relevance: The pilot study provides evidence that during routine gastroscopy it is possible to sample endoscopic biopsies from the cardia and with limitations from the esophagus showing a quality adequate for histological examination of the epithelium and the lamina propria mucosae.

Schlüsselwörter
Ösophagoskopie, Histologie, Speiseröhre, Magen, Z-Linie, Biopsietechnik

Zusammenfassung
Introduction

Today endoscopy of the canine esophagus, stomach, and duodenum is clinically well established (18, 46). Biopsy and histological examination of the stomach and duodenum have become a closed part of this diagnostic procedure (7). In contrary, biopsy and histological examination of the esophagus are not routinely performed in upper gastrointestinal endoscopy. The esophageal biopsy procedure has been mostly hindered by anatomic reasons (20). The natural resistance of the epithelium and the tight lamina propria mucosae of the canine esophagus hamper obtaining specimens valid for histological examination (18). Because of the tubular structure of the esophagus forceps are often orientated in adverse angels to the mucosal surface further aggravating biopsy sampling. The use of rigid forceps, suction biopsy or capsule systems may lead to better results (44), but does not represent methods acceptable for routine gastroscopy. Consequently, because esophageal samples are rarely endoscopically obtained, veterinary clinical pathology is not very experienced in this specimen processing and histological examination.

In humans, during the last 15 years esophagoscopy, biopsy and histological examination of the esophagus has become a standard procedure because of a high incidence of esophageal disorders. One of the most common gastrointestinal disorders in humans is gastro-esophageal reflux disease (GERD) (42), in which pathologic gastro-esophageal reflux causes heartburn, regurgitation, and endoscopic and histological lesions at the esophagus and the gastro-esophageal junction (GEJ). Pathologic gastro-esophageal reflux can lead to esophageal complications such as esophagitis, stricture formation, and intestinal metaplasia (Barrett’s esophagus), and to respiratory complications (5). Several endoscopic grading systems such as the Los Angeles classification system or the Prague classification system have been established to standardize the diagnosis of GERD (3, 29) and Barrett’s esophagus (35), respectively. To endoscopic biopsy of the esophagus several techniques such as the turn-and-suction technique (24) have been developed, and the use of several forceps has been recommended such as disposable and reusable forceps (37), standard forceps, large capacity forceps, and jumbo forceps (8, 17). Specimens were recommended to be preferably obtained exactly from the GEJ or 2 cm proximal by some investigators (45), but all available endoscopic classification systems do not give a recommendation when and where to take a biopsy in the distal esophagus and consensus is recommended (40). Reported esophageal specimen sizes leading to adequate histological results showed preferably > 1.5–2 mm mean width, depending on the used forceps (17, 24). Histological findings such as basal cell hyperplasia, papillary elongation, inflammation, and dilated intercellular spaces of the esophageal mucosa are reported from patients with GERD, and histological standardizations are achieved (2, 12).

The incidence of GERD in dogs is unknown. However, it could be shown, that dogs can suffer from multiple structural lesions in the area of the terminal esophagus and the cardia, either induced experimentally (9, 10, 36, 38), or due to natural disease (1, 11, 14, 15, 22, 25, 26, 32, 33, 39). Reported lesions possibly caused by pathologic gastro-esophageal reflux such as esophagitis or intestinal metaplasia are histological defined diseases (15, 16, 19). Their true incidence is unknown but it has been assumed that underestimation is possible because symptoms and lesions may be overlooked, and histology is rarely applied (21). Histological examination of this area represents an essential tool for qualified diagnostics. Therefore, a pilot study was performed to review that diagnostic option. Working hypothesis of the study was that specimens obtained from the canine gastro-esophageal junction during routine gastroscopy are valid for histological examination.

Materials and methods

The aim of the study was to assess histological quality of biopsy specimens obtained from the canine gastro-esophageal junction. For this purpose 10 dogs were examined, that had been presented to a referral practice between November 2010 and February 2011 because of the presence of gastro-intestinal symptoms indicating gastroscopy. At the minimum clinical investigations encompassed signalment, history, clinical examination, endoscopic examination of the esophagus, endoscopic biopsy, and consecutive histological examination.

Esophagoscopy and biopsy

Esophagoscopy was performed by using a flexible endoscope (Olympus GIF-PQ20). Food had been withheld for a minimum of 16 hours before examination. After anaesthesia with acepromazine (0.02–0.04 mg/kg bodyweight [BW] intramuscularly, Vetranquil®)
and propofol (2–4 mg/kg BW intravenously, Narcofol®) dogs were positioned in left lateral recumbency. Esophagoscopy was documented by use of a standardized protocol (www.vetgastro.de). Biopsies were performed by application of standard forceps manufactured for single use in man with a working diameter of 2.3 mm, elliptic branches, and a lancet (MTW-Endoskopie®, Wesel; article number 99063502815). Hereto per dog a minimum of five specimens were obtained exactly from the junction, where the pale esophageal mucosa changes to the red cardiac mucosa. Specimens were preferably taken from the dorsal and right esophageal wall and from the cardia of the greater curvature (Fig. 1).

**Histology**

Samples from the individual dog were divided in equal numbers, fixed in 4% neutral buffered formalin, and given to transportation containers. For further routine processing (paraffin embedding, cutting, and haematoxylin and eosin staining) containers were sent to two different laboratories (laboratory for veterinary pathology: Synlab.vet, Köln; laboratory for human pathology: Institut für Pathologie, Sankt Elisabeth Krankenhaus Hohenlind, Köln, respectively). Histological examinations were performed by a veterinary pathologist, and a pathologist for human medicine, respectively. Specimens were judged by the pathologists in a not blinded case discussion after all cases had been sampled.

Histological quality was estimated by following criteria: presence or absence of specimens from the esophagus defined as stratified squamous epithelium with basal cell layer and lamina propria mucosae (Fig. 2), and presence or absence of specimens from the cardia containing columnar epithelium, defined as foveolar epithelium in combination with basal mucoid glands showing no or only sparse fundic glands (Fig. 3); presence or absence of specimens showing at least two layers (epithelium and lamina propria mucosae) originating from the esophagus, and the cardia, respectively; presence or absence of at least one specimen with a diameter of > 2 mm on the slide originating from the cardia, and from the esophagus, respectively (17, 41).

The primary outcome of the study was adequacy of biopsies for histological assessment. An adequate biopsy was defined as specimens in adequate size (> 2 mm), originating exactly from the gastro-esophageal junction, and showing at least two layers from esophageal and cardiac mucosa. For this purpose, dogs reaching

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Fig. 2 Histological picture of an adequate endoscopic biopsy specimen from the canine esophagus showing stratified squamous epithelium (E) with basal cell layer (BC), and lamina propria (P) with papillae (PAP). (HE, 100×).

Abb. 2 Histologisches Bild eines adäquaten endoskopischen Bioptats aus dem kaninen Ösophagus: mehrschichtiges Plattenepithel (E) mit Basalzellschicht (BC) und der Lamina propria (P) mit Papillen (PAP). (HE, 100×).

Fig. 3 Histological pictures of adequate endoscopic biopsy specimens from the canine cardia (3a) and corpus (3b). Cardia: simple columnar epithelium with cardiac glands: short, coiled, and basal mucoid tubular glands (A). Transition to the proper gastric gland region (B): occurring of parietal cells (arrows). Corpus: simple columnar epithelium with proper gastric glands (C): long, straight tubular glands. (HE, 40×).

this endpoint (Table 1) were estimated, and specimens of these dogs were found to be adequate for histological assessment.

**Statistical analysis**

For statistical analysis breed, age, gender, bodyweight, symptoms, endoscopic findings, and number of biopsies were recorded at first. The presence of Gaussian distribution for quantitative data was tested by Kolmogorov-Smirnov-Test. Therefore, age, body weight, and number of biopsies were given as median and range. Next, numbers of dogs were estimated that had shown the histological quality criteria as described above. Next, numbers of dogs were recorded reaching the endpoint as described above. All data were given descriptively in absolute and relative numbers. Analysis was supported by the use of a commercially available computer package (WinSTAT® für Excell, R. Fitch Software, In der Breite 30, D-79189 Bad Krozingen).

**Results**

**Clinical findings**

Included dogs comprised 3 shepherd dogs (1 German shepherd dog, 1 Belgian shepherd dog, 1 Australian shepherd dog, respectively), 3 mixed-breed dogs, 1 Golden retriever, 1 Irish terrier, 1 Hovawart, and 1 Newfoundland dog. Body weight ranged from 8.0 kg to 50.0 kg (median 27.5 kg), the age varied between 1.0 year and 11.0 years (median 4.0 years). Three of 6 male dogs and 3 of 4 female dogs were neutered, respectively. Symptoms indicating gastroscopy were present in all dogs. Chronic vomiting in 4 of 10 (40.0%) dogs, regurgitation in 3 of 10 (30.0%) dogs, and chronic small bowel diarrhoea in 6 of 10 (60.0%) dogs were the reported symptoms.

**Esophagoscopy and biopsy**

No obvious alterations were reported from the lumen, the containment, the elasticity of the wall, and from the mucosal surface of the esophagus. The gastro-esophageal junction was identified in all dogs. Indications of alterations of the gastro-esophageal junction were found in 6 of 10 (60.0%) dogs. The only finding was erythema of the esophageal mucosa and a mildly increased friability of the mucosa at the gastro-esophageal junction. No dog had shown surface defects such as erosions or confluent mucosal breaks. In every dog multiple excisions had been performed. For this purpose during esophagoscopy the tip of the endoscope with the opened forceps was brought into a mostly vertical position onto the mucosa of the dorsal esophagus, the gastro-esophageal junction, and the cardia of the greater curvature. Then the forceps was a little advanced to the mucosa while the endoscope was fixed manually in front of the mouth to prevent a sliding back. Finally, the branches were closed and the forceps was drawn on a short way back into the endoscope. Specimens obtained from the esophagus cranially to the gastro-esophageal junction often were macroscopically smaller in size, and in most cases biopsies had to be repeated for several times, until acceptable sizes were recruited. The operator estimated specimen’s size as following: the forceps was opened in a test tube filled with physiologic saline solution flipping the tip against the wall. The swimming piece became visible and macroscopic size was estimated by the eye, initially comparing it with the tip of the forceps. Preferably pieces estimated >2 mm were given to the container.

**Histology**

In 10 dogs 72 specimens (median 7, range 5–10) from the gastro-esophageal junction had been examined on the slides. Histological quality criteria were assessed by both pathologists by common evaluation of the pooled specimens (Table 2). At least epithelium and lamina propria mucosa were present in specimens from the esophagus in 5 of 10 (50.0%) dogs, in 3 of 10 (30.0%) dogs specimens were found to be too superficial, and in 2 of 10 (20.0%) dogs squamous epithelium was not present. At least epithelium and lamina propria mucosa were present in specimens from the cardia in 10 of 10 (100.0%) dogs, and in 3 of 10 (30.0%) dogs corpus mucosa was present as well. In 10 of 10 (100.0%) dogs and in 4 of 10 (40.0%) dogs one or more specimens showed >2 mm on the slide, originating from the cardia, and from the esophagus, respectively. In 7 of 10 (70.0%) dogs, columnar epithelium and squamous epithelium were found on the same slide (Fig. 4). Quality was found to be adequate in 4 of 10 (40.0%) dogs, showing specimens originating exactly from the gastro-esophageal junction (at least >2 mm, presence of both sites and two layers).

Despite diagnostic judging was behind the scope of the study, the pathologists tried to judge the dogs. The dogs were found to have normal esophageal and cardiac mucosal findings or findings such as hyperaemia, oedema, cellular infiltrates, foveolar hyperplasia, and fibrosis.
Discussion

The aim of the study was to test the adequacy of biopsies sampled during routine gastroscopy from the gastro-esophageal junction. For this purpose dogs presented for gastroscopy were included into the study. Reported symptoms such as chronic vomiting, regurgitation, and chronic small bowel diarrhoea had indicated gastroscopy (46). Dogs of both gender in a spectrum of body weights between 8 and 50 kg and an age between 1 and 11 years were examined. Random sample was small, dogs < 8 kg, and dogs < 1 year were not represented.

Anatomically, at the gastro-esophageal junction the esophagus bridges into the ostium cardiacum. The latter is composed of a pars proventricularis characterized by cutaneous mucosa and the following small cardiac zone characterized by foveolar mucosa. The cutaneous mucosa of the pars proventricularis is coloured whitish, smooth, and tough, carrying squamous epithelium on a prominent papillary body. It is sharply contrasted against the columnar epithelium of the red foveolar mucosa (34). Endoscopically this area is called z-line in man, while in veterinary endoscopy the term gastro-esophageal junction is preferred (18). The junction is located 1.5 cm cranially to 0.5 cm caudally to the hiatus esophagi of the diaphragm (31). Histologically, the junction is mostly abrupt but can also show a width of 1 mm or more millimetres in the dog (6). The cardia is found as a small strip of 1–2 cm caudally to the junction (13), with foveolar epithelium defined as simple columnar epithelium with cardiac glands: short, simple, branched, coiled, and basal mucoid tubular glands, expecting no or sparse fundic glands in this area (28).

Endoscopically, the gastro-esophageal junction was identified in all dogs of our study. Recognizing is possible with a fiberscope as used for the study (30). The endoscopic landmark is the junction, where the colour changes from pale to red, and identifying of the esophageal mucosa is easy. In dogs the extension of the cardiac mucosa is small, and endoscopic differentiation between cardiac and corpus mucosa is not possible. Therefore, in biopsy procedures cardiac mucosa may be missed, and just the transition area of cardia to corpus, or even corpus may be hit, limiting the adequacy of the biopsy. As well, esophageal or cardiac mucosa may be missed in biopsies, when the change in mucosal colour may be endoscopically not that obvious, or even when panting or pathological conditions are present.

### Table 2

Results of the histological quality assessment of biopsies from the gastro-esophageal junction in 10 dogs.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Specimen (n)</th>
<th>Squamous epithelium with lamina propria</th>
<th>Esophageal specimen &gt; 2 mm</th>
<th>Columnar epithelium with lamina propria</th>
<th>Cardiac specimen &gt; 2 mm</th>
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**Fig. 4** Histologic picture of a biopsy specimen from the gastro-esophageal junction: stratified squamous epithelium of the esophagus (left) and simple columnar epithelium of the cardiac gland region (right) present in one biopsy. Note the abrupt junction (arrow). (HE, 100×).

**Abb. 4** Histologisches Bild eines Biopatts vom gastroösophagealen Übergang: mehrschichtiges Plattenepithel des Osophagus (links) und einschichtiges Zylinderepithel aus der Kardiadrüsenzone (rechts) in einer Probe. Auffällig ist der abrupte Übergang (Pfeil). (HE, 100×).
ditions may hinder exact localization of the gastro-esophageal junction. In three dogs of our study biopsies from both corpus and cardia were found in one container. Just when biopsies originated from the cardia or from the transition area they had been judged as cardiac biopsies. At least one specimen from these origins was found in every dog of our study.

Before the study was started, the described biopsy technique had been found empirically to be the best applicable for sampling specimens from the junction. Specimen numbers varied between 5 and 10 per dog to ease histological examination in cases of suspected lower quality, or in cases of suspected failed localization such as corpus. The preferably vertical endoscopic biopsy position, the pressure on the biopsy forceps, the use of forceps with a lancet, and the sharpness of the unused branches may have contributed to the sample qualities of our cases (4). Improvement of quality by use of other biopsy techniques (27) or so called large capacity forceps (24) particularly contributed to better results in man, and remain to be proven in dogs. Minimal endoscopic lesions such as erythema and mildly increased friability had been reported from 6 dogs in our study, but esophagitis was not confirmed by histology. These lesions without obvious mucosal breaks are mostly classified as normal findings in human endoscopy (40). Minimal findings such as small erosions, an atypical z-line, changes in vascular architecture, and others have been demonstrated in man using magnifying endoscopy (23), representing lesions that could not be recognized in our study because of the use of a fiberscope.

In our study the criteria for the assessment of histological quality of biopsies from the gastro-esophageal junction had been adapted from human studies (17, 41) because to our knowledge they are not reported in the veterinary literature. Therefore in the individual dog we assessed the site, length, depth, and adequacy for diagnostic judging from the best available specimens. Adequate depth, the presence of both epithelium and lamina propria mucosae was reached in esophageal pieces in 50% of the dogs, and a length of 2 mm and greater was achieved in the esophagus in 40% of the dogs. In general, the biopsy yielded smaller samples from the esophagus than from the cardia. Several causes may have contributed to a lesser size in esophageal specimens. The predominant superficial character of the esophageal specimens was ascribed to the known rough epithelium and tight substratum of the carnivorous esophageal mucosa (18). As well, empirically, biopsy samples can show shrinkage of up to 50% of size when they were fixed in formalin. Furthermore, specimens processed in the laboratory for human pathology were more often slightly better orientated on the slides compared to those processed in the laboratory for veterinary pathology. This quality difference was thought to be the less associated to randomly varying sampling qualities, but to different degree of experience in handling samples from the esophagus in the laboratories. Similar experiences are reported from a study that compared histological biopsy results in dogs between different institutions from other sites of the gut (43). Concerning to the endpoint criteria, 40% of the cases of our study reached a specimen quality adequate for histological examination, what seems to be low. In man under similar parameter setting (pieces ≥ 2 mm, layers with at least lamina muscularis mucosae present, and specimens well orientated), an adequate quality is achieved in 33%, 38%, and 28% of the cases, depending on the biopsy technique used (standard forceps, large capacity forceps, and jumbo forceps, respectively) (17).

Conflict of interest

The authors confirm not to have any conflict of interest.

References


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